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Detection of loci affecting milk production and health traits in an elite US Holstein population using microsatellite markers

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Summary

Quantitative trait loci (QTL) affecting health and milk production traits were studied in seven large half-sib US Holstein families by using the granddaughter design. Genotyping for 16 markers was completed and marker allele differences within and pooled-across families were analysed. Potential QTL were identified for somatic cell score (SCS), fat yield, fat percentage, protein yield and protein percentage. Three markers (BM203, BM4505 and BM2078) were associated with significant effects for different traits and, after further analysis, may be useful in marker-assisted selection in specific families. Comparisons between these data and previously identified QTL support the location of a QTL for milk yield and protein yield on chromosome 21.

Keywords: dairy cattle, microsatellite markers, milk production, quantitative trait loci, somatic cell score

Introduction

Genetic progress in dairy cattle has been based on progeny testing of potential sires. While progeny testing has been successful, it requires approximately five years to conduct the testing. Alternatives or supplements to progeny testing would be useful in improving selection accuracy and reducing the generation interval.

Marker-assisted selection (reviewed in Soller 1994), using genetic marker information in selection programmes, was proposed as one such alternative; however, at that time, few bovine genetic markers were available. Since the discovery of microsatellite markers and development of bovine linkage maps (Barendse *et al.* 1994; Bishop *et al.* 1994), investigation of markers, which are linked to genes affecting quantitative traits, has become feasible. Characterization of quantitative trait loci (QTL) for economically important traits may lead to more efficient breeding programmes using marker-assisted selection, especially for bulls prior to progeny testing.

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Prior to the availability of abundant microsatellite markers, a candidate gene approach was used to identify QTL for milk production traits. Allelic variants of blood groups, β-lactoglobulin, κ-casein and β-casein, as well as other genes (Neimann-Sorenson & Robertson 1961; Geldermann *et al.* 1985; Cowan *et al.* 1990, 1992; Andersson-Eklund & Rendel 1993; Bovenhuis & Weller 1994; Velmala *et al.* 1995), were shown to be associated with chromosome substitution effects for many milk traits. However, many grandsire families were uninformative (owing to the diallelic nature of the polymorphisms) or may have been homozygous at the QTL, so other QTL-milk production trait associations were not detected. Microsatellite markers, on the other hand, are frequently highly polymorphic, making them useful in mapping studies. Recent reports have used microsatellite markers located throughout the genome to identify QTL for health and milk production traits (Ron *et al.* 1994; Georges *et al.* 1995; Weller *et al.* 1995).

Initial microsatellite studies investigated QTL that affected milk production traits. Ron *et al.* (1994) used 10 microsatellite markers to search for QTL that affected milk production traits, in seven Israeli Holstein families, using the granddaughter design (Geldermann 1975; Weller *et al.* 1990). Results from this study identified one marker (D21S4) that was associated with significant effects on milk and protein yields in one family.

Georges *et al.* (1995) carried out a larger study, searching for markers linked to QTL in 14 US Holstein half-sib families. Using 159 microsatellite markers, milk production QTL were detected on five chromosomes (1, 6, 9, 10 and 20) with various effects on milk yield, protein yield and percentage and fat yield and percentage.

More recently (Weller *et al.* 1995), secondary traits with lower heritabilities have been studied using the Dairy Bull DNA Repository (DBDR; Da *et al.* 1994). Eleven microsatellite markers were selected for genotyping sires, from 15 DBDR families, to identify significant effects for somatic cell score (SCS), productive herdlife and milk production traits. Five markers were

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associated with significant effects in several families, with some markers being important for more than one trait.

This report describes preliminary results using 16 microsatellite markers, genotyped in seven large Holstein families, to identify potential QTL associated with seven economically important traits – milk yield, protein and fat yield, protein and fat percentage, productive herdlife and SCS.

Materials and methods

Source of DNA

Semen samples were selected from the DBDR (Da *et al.* 1994), located at the University of Illinois. The DBDR is a collection of semen from 35 half-sib families in the granddaughter design (Weller *et al.* 1990). Seven large families were selected for this study; selection was based on the number of available sons' semen and the number of daughters with milk somatic cell information represented by each son (greater than 50 daughters per son). DNA was isolated from approximately 900 US Holstein bulls by using a lysis/phenol-chloroform protocol described previously (Ashwell *et al.* 1996).

Microsatellite markers

Seventy-seven microsatellite loci, located throughout the genome, have been selected for genotyping. Selection of these loci was based on the number of observed alleles and the location of the markers on each bovine autosome; marker spacing was 20–50 cM whenever possible. Here we report the results from 16 of these markers (Table 1; selection criteria described in Results). Marker information, including polymerase chain reaction (PCR) annealing temperatures, primer sequences, and linkage map locations, was previously reported by Bishop *et al.* (1994). All primers were identical to those described in Bishop *et al.* (1994) with the exception of the BM2078 forward primer. A new forward primer was designed because the original PCR produced no amplification product with a fluorescently tagged forward primer. The BM2078 forward primer used in this study was 5'-CAGACTCTGAGCCCAAAAG-3', making the PCR product 11-bp longer than the original PCR amplification product (using the same PCR conditions and annealing temperature; see Bishop *et al.* (1994).

Table 1. Microsatellite markers genotyped for all available sons of seven selected Dairy Bull DNA Respository (DBDR) families

Chromosome	Locus	No. of alleles	No. of heterozygous grandsires	No. of sons genotyped	No. of informative sons (%)
2	BM4440	4	4	468	341 (73)
8	BM711	4	7	820	613 (75)
9	BM4204	3	4	583	356 (67)
14	BM302	6	5	503	433 (86)
18	BM2078	5	4	389	323 (83)
21	BM103	6	7	799	619 (78)
21	BM3413	3	5	622	425 (68)
22	BM3628	6	5	551	458 (83)
23	513*	5	5	617	438 (71)
23	BM1258	4	3	312	251 (80)
23	BM1443	5	4	353	286 (81)
23	BM1818	3	4	449	276 (61)
23	BM1905	4	5	477	337 (71)
23	CYP21	3	4	431	281 (66)
26	BM4505	6	5	559	513 (92)
27	BM203	5	6	643	495 (77)

*Data reported in Ashwell *et al.* (1996).

PCR and gel electrophoresis

PCR was performed using either radioactive or fluorescent methods. Fifty nanograms of genomic DNA was aliquoted into 96-well microtitre plates and amplified in the presence of 1·5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9·0), 30 µM each of unlabelled dCTP, dGTP and dTTP, 3·0 µM dATP, 0·1 µCi [α-^{32}P]dATP, 0·4 µM each primer and 0·35 units *Taq* DNA polymerase, in a total volume of 12 µl. Fluorescence PCR was performed as above but with 30 µM each of unlabelled dCTP, dGTP, dTTP and dATP, and 0·4 µM of a fluorescently tagged forward primer and unlabelled reverse primer. The Hybaid Omnigene (Middlesex, UK) thermal cycler protocol was as follows: 94°C for 3 min followed by 30 cycles of 1 min at 94°C, 1 min at the annealing temperature (see Bishop *et al.* 1994) and 1 min at 72°C followed by a final extension step of 5 min at 72°C. The MJ Research DNA Engine (Watertown, MA) thermal cycler protocol was similar to the Hybaid protocol except each step was reduced from 1 min to 15 s. PCR products were separated on a 6% denaturing polyacrylamide gel and exposed to film overnight (radioactive PCR) or analysed on an ABI 373 Stretch Automated Sequencer (fluorescence PCR; Foster City, CA).

Data collection and analysis

Marker allele differences for seven traits (SCS, milk yield, protein yield, fat yield, fat percentage, protein percentage and productive herdlife)

were tested within and pooled-across sire families. The mixed models program PROC MIXED of SAS (SAS Institute 1996) was used to evaluate the data in the granddaughter design. Weller *et al.* (1990) estimated that the power to detect a gene effect of 0·2 genetic standard deviations would be 67% for traits such as SCS and herdlife ($h^2 = 0{\cdot}1$), 48% for milk, fat and protein yields ($h^2 = 0{\cdot}2$) and 21% for fat and protein percents ($h^2 = 0{\cdot}5$) from a population of five heterozygous grandsires with 100 sons/grandsire and 50 daughters/son. These estimates are conservative because our population was slightly larger and we used $P = 0{\cdot}05$ instead of 0·01 as significance for type I error.

Daughter records for each son from the January 1996 animal model evaluation (VanRaden & Wiggans 1991) were combined into daughter yield deviations (DYD) and daughter SCS deviations (DSD) by the animal model (VanRaden & Wiggans 1991). Single marker-allele differences were analysed within each grandsire family using the model

$$DD_{ij} - 0{\cdot}5\ DPTA_{ij} = ALLELE_i + ERROR_{ij}$$

where DD_{ij} is the DSD or DYD for the j^{th} son that inherited the i^{th} marker allele from the grandsire and $DPTA_{ij}$ is the dam's predicted transmitting ability. The pooled-across family analyses were conducted with the model

$$DD_{ijk} - 0{\cdot}5\ DPTA_{ijk} = GSIRE_i + ALLELE_{ij} + ERROR_{ijk}$$

where $GSIRE_i$ is the effect of grandsire i. Observations were weighted by the son's reliability, which is proportional to the reciprocal of the variance of DYD. If reliability included only daughter information, and not parental information, weights for DYD would be more precise. The marker-allele difference estimates the QTL allele difference multiplied by (1–2r), where r is the recombination rate between marker and QTL. A significant marker difference implies the presence of a segregating QTL. The 0·05 level was selected as the probability level for the initial analysis to ensure inclusion of possible QTLs with the recognition that the true number of significances, on a per experiment basis, would be fewer than those generated on a per comparison basis.

Because chromosome 23 was covered by six markers, interval mapping (Lander & Botstein 1989) was also conducted for each of the seven traits using the ANIMAP programs (Georges *et al.* 1995).

Table 2. Significant marker-allele differences pooled across families

Locus	Chromosome	Trait	Across-family significance
BM2078	18	SCS	0·010
		Fat yield	0·032
BM3413	21	Milk yield	0·021
		Fat yield	0·039
513	23	Fat yield	0·015
BM1258	23	Herdlife	0·039
BM4505	26	Fat yield	0·011
		% Fat	0·041
BM203	27	Protein yield	0·029
		% Protein	0·020

Results

Marker genotyping

Seventy-seven markers have been selected for genotyping in seven Holstein families to complete a preliminary QTL scan of the genome. Here we report the results from 16 of those microsatellite markers. Table 1 provides genotyping information on these markers. Selection of the markers was based on several criteria. Six markers were selected on chromosome 23 as previously reported in our study (Ashwell *et al.* 1996) that described the association of marker 513 with the SCS trait. Genotyping of additional markers located on this chromosome was conducted in an attempt to localize more precisely the previously detected QTL. Four additional markers (BM4204, BM302, BM2078 and BM3628) were selected, based on their possible associations with QTL for SCS, by using selective genotyping (Darvasi & Soller 1992) (data not shown). The remaining markers were chosen based on the availability of fluorescently tagged primers and the PCR product size, for use on the ABI 373 Automated DNA Sequencer. Based on data from the 16 markers, approximately four of the seven grandsires were heterozygous at each marker. An average of 77% of the sons from heterozygous sires were informative, i.e. the transmission of the grandsire allele could be determined. Therefore, the number of informative sons was fewer than the total number of sons.

Significant marker allele differences

Significant across-family effects by locus and trait are given in Table 2. Six markers were associated with significant effects ($P < 0{\cdot}05$) for different traits. Four markers, BM4505, BM3413, BM2078 and BM203, were associated with significant effects ($P < 0{\cdot}05$) for more than one trait. As these effects were in some of the same families and affected related traits, such as protein yield and protein percentage, it is probable that

the effects on both traits were caused by a single segregating QTL. Significant ($P < 0{\cdot}05$) within-family effects are given in Table 3 by locus and trait using a single-trait analysis. Several effects associated with single traits (e.g. BM302, in family 8, affecting SCS and BM4505, in family 4, affecting fat yield) were highly significant ($P < 0{\cdot}01$) and these effects should be confirmed with further testing.

BM3413 on chromosome 21 was associated with significant effects on three traits in family 5. In this family, one allele was associated with significant increases in milk yield, protein yield and fat yield.

Marker 513 on chromosome 23 was associated with marker-allele differences for several traits in two families. In family 1, one allele was associated with increased fat yield, fat percentage and herdlife. In family 9, one allele was associated with decreased SCS, increased milk yield and significant increases in fat yield.

BM2078 on chromosome 18 was associated with significant effects for several traits in two families. In family 4, one allele was associated with a higher SCS and a lower fat percentage and yield. In family 8, three significant effects were observed: milk yield was dramatically reduced for one allele, SCS and protein yield were also reduced.

Allele 225 (referring to the number of base pairs for the allele) at marker BM203 appeared to be associated with a consistent increase in protein yield in all families carrying that allele, with an average increase of 2·81 kg of transmitting ability for protein (Table 4). This is the only specific marker allele for which a consistent positive or negative effect was found.

Interval mapping was used to identify QTL on chromosome 23 using data from six microsatellite markers. The most probable order of the markers is BM1443–BM1905–BM1818–CYP21–513–BM1258 with 3, 11, 11, 5 and 10 cM between the markers, respectively (Bishop *et al.* 1994; http://sol.marc.usda.gov). Two potential QTL had LOD scores greater than 1·45, which is equivalent to a P value of approximately 0·01 (Ott 1991). In family 4, a potential QTL for somatic cell score was detected between CYP21 and BM1818 (5 cM from the BM1818 marker), with a LOD score of 2·046. Using the single-marker analysis results, we detected a potential marker-QTL association for SCS in family 4 with marker 513, BM1905 and the strongest evidence occurring with BM1818 ($P = 0{\cdot}0049$). In family 12, potential QTL for protein and percentage protein were detected, but at slightly different locations. Using the protein data, the QTL is located between BM1818 and BM1443, 10 cM from BM1443, with a LOD score of 1·821. Using the percentage protein data, the QTL is located between the same two markers but 2 cM from BM1443, with a LOD score of 1·554. Using the single-marker analysis findings, we detected a potential marker-QTL association for percentage protein in family 12 with BM1443 and BM1905. A similar association for protein yield was not detected using the single-marker analysis.

Discussion

This paper reports potential marker-QTL associations for seven economically important traits, using 16 microsatellite markers, in a Holstein cattle population. All markers except BM4440, BM3628 and BM4204 were associated with significant effects for at least one trait in one family. Theoretically, more significant marker differences would be found in grandsire families with larger variances. However, our results are not in good agreement with this theoretical expectation. For example, of four significant differences with $P < 0{\cdot}01$ in Table 3, one significant difference was found in the family with the largest variance, one was found in the family with the third largest variance and two were found in the families with the second smallest variance. This deviation from the theoretical expectation could be explained by the fact that a percentage of the offspring were non-informative and this resulted in incomplete usage of the trait data in the family.

The majority of the effects identified were associated with the milk production and composition traits; however, several markers were associated with potential QTL for SCS and productive herdlife. A total of 48 significant effects were identified in the within-family analysis although 26 of these might be explained by chance at $P < 0{\cdot}05$ because 515 significance tests were performed. Caution should be used, however, when evaluating these marker-QTL associations because of the large number of significance tests that were performed. Many associations may be due to chance, giving too many false positive claims if too lax a linkage standard is used. However, if too strict a guideline is used, many QTL may go unreported.

Lander & Kruglyak (1995) calculated critical values to account for multiple testing over the entire genome in order to avoid large numbers of false positive claims of linkage. They stated the need for genome-wide threshold values, where the P value needed to be between 10^{-4} and 10^{-5} for significant linkage and 10^{-3}–10^{-4} for suggestive linkage. None of the P values we report are of the appropriate order of magnitude for sug-

Table 3. Within-family significant marker allele differences (by locus and trait)

Locus	Chromosome	Trait	Family	*P*	Marker allele difference*	SE
BM711	8	Protein yield	9	0·0404	3·95	1·92
		% Protein	12	0·0133	–0·034	0·014
		% Fat	3	0·0296	–0·038	0·017
BM302	14	SCS	8	0·0096	0·13	0·048
		Milk yield	5	0·0302	119	55
		% Fat	1	0·0181	–0·035	0·015
BM2078	18	SCS	4	0·0220	0·087	0·038
		SCS	8	0·0106	–0·12	0·046
		Milk yield	8	0·0058	–191	69
		Protein yield	8	0·0164	–4·68	1·94
		Fat yield	4	0·0080	–5·57	2·09
		% Fat	4	0·0360	–0·040	0·019
BM103	21	Herdlife	3	0·0378	0·82	0·39
		% Protein	5	0·0438	0·019	0·009
		% Fat	8	0·0315	0·056	0·026
BM3413	21	Milk yield	5	0·0013	201	62
		Protein yield	5	0·0122	4·33	1·72
		Fat yield	1	0·0182	–3·52	1·48
		Fat yield	5	0·0141	6·00	2·43
513	23	SCS	4	0·0445	–0·083	0·041
		SCS	9	0·0410	–0·11	0·05
		Milk yield	9	0·0149	172	70
		Herdlife	1	0·0391	0·68	0·33
		Fat yield	9	0·0098	7·11	2·74
		Fat yield	1	0·0101	4·55	1·76
		% Fat	1	0·0193	0·040	0·017
BM1258	23	Herdlife	1	0·0222	0·72	0·31
		Fat yield	1	0·0380	3·27	1·57
BM1443	23	SCS	3	0·0137	0·085	0·034
		Protein yield	8	0·0482	3·13	1·58
		% Protein	12	0·0271	0·030	0·014
BM1818	23	SCS	4	0·0049	0·108	0·038
BM1905	23	SCS	4	0·0418	0·078	0·038
		SCS	3	0·0333	0·076	0·035
		% Protein	12	0·0267	–0·032	0·014
		% Fat	9	0·0470	–0·048	0·024
CYP21	23	Herdlife	9	0·0317	–1·61	0·74
		% Fat	1	0·0253	0·038	0·017
BM4505	26	SCS	3	0·0128	–0·078	0·031
		Fat yield	4	0·0037	5·59	1·92
		Fat yield	9	0·0428	4·85	2·39
		% Fat	5	0·0433	0·032	0·016
BM203	27	Milk yield	8	0·0210	–174	76
		Protein yield	4	0·0290	3·50	1·60
		Protein yield	8	0·0499	–4·10	2·08
		% Protein	1	0·0288	0·017	0·008
		% Protein	5	0·0218	–0·019	0·008
		% Fat	8	0·0476	0·054	0·027

*Units of marker-allele differences: milk, fat, protein yield reported in kg; SCS (somatic cell score) adjusted to log base 2 of the concentration; % protein and fat reported as % of protein or fat yield/milk yield; herdlife reported as months of life, limited to 7 years 10 months of life/lactation.
P, probability (estimated by using the mixed models program PROC MIXED.
SE, standard error.

gestive or significant linkage using the threshold of Lander & Kruglyak (1995). However, QTL detection in dairy cattle is at an exploratory stage and Lander & Kruglyak (1995) state that it is important to report all regions with $P < 0{\cdot}05$ but not to make conclusive linkage claims.

While Lander & Kruglyak (1995) believe that their standards are appropriate, others believe the levels of significance to be too strict. Witte *et al.* (1996) objected to Lander & Kruglyak's (1995) suggestion to report only one or a few extreme *P* values, and suggested that all *P* values, obtained from a genome screen, should be plotted. Curtis (1996) also objected to Lander & Kruglyak's (1995) criteria and suggested that the subject of which results should be published should continue to be a matter for negotiation and informed discussion. We completely agree with the opinion that false positive results should be avoided and we also agree that the criteria for reporting results should not be so restrictive that it becomes virtually impossible for many research projects to report results. We expect that concurrence among several studies will be the most useful indicator of true QTL locations.

Three markers in this report were associated with several effects that warrant additional study.

(1) Allele 225 of BM203 in families 1, 4, 5 and 8 may prove useful in increasing protein yield. Additional gain may be achieved with allele 225 in family 8, with the potential for increased milk yield in addition to the increase in protein yield.

(2) BM4505 on chromosome 26 may prove useful in family 3 because one allele was associated with increased protein yield and reduced SCS. In other families, alleles for this marker may be useful in varying fat yield and percentage with little or no effect on other evaluated traits.

(3) BM2078 appeared to be a very promising marker based on the selective genotyping data. After complete genotyping, the marker still appeared to be associated with a QTL for SCS. However, after examination of the within-family results, BM2078 may not be very useful in marker-assisted selection. In family 8, one allele was associated with decreased SCS, as well as decreased protein and milk yield, thus supporting the reported genetic antagonism between milk yield and mastitis resistance (Emanuelson *et al.* 1988).

Table 4. Within-family analysis of marker BM203 with protein yield

Family	Alleles	Marker allele difference	SE	*P*
1	225, 231	1·96	1·25	0·117
4	225, 231	3·50	1·60	0·029
5	225, 209	1·68	1·32	0·203
8	225, 217	4·10	2·08	0·049
9	217, 231	0·30	2·17	0·889
12	221, 231	2·85	2·39	0·233

P, probability (estimated by using the mixed models program PROC MIXED).
SE, standard error.

Our studies may confirm the QTL detected by Ron *et al.* (1994) in an Israeli Holstein family. In this study, strong evidence of a QTL for milk yield and protein yield near D21S4 (ETH131) was reported. In our study, DBDR bulls were genotyped at two markers on chromosome 21: BM103 and BM3413. With marker BM3413, we detected marker-allele differences that affected milk yield, protein yield and fat yield in one family. Based on the estimated effects, it is probable that we are detecting the same QTL as reported by Ron *et al.* (1994).

Georges *et al.* (1995) studied 14 US half-sib elite pedigrees. Although none of the grandsires were identified, it is probable that some of the families in that study and in ours are the same. Except for chromosome 9, we have not genotyped markers on chromosomes where Georges *et al.* (1995) detected QTL. Using our BM4204 data we detected no QTL for milk yield and are unable to confirm this QTL in our families.

Recently, Weller *et al.* (1995) used microsatellite markers to study DBDR families, identifying potential QTL for seven health and milk production traits. We have genotyped one marker on chromosome 2, where Weller *et al.* (1995) detected a potential QTL for fat yield and percentage. We detected no significant effects in four families, with two families being identical between the two studies. However, as the marker of Weller *et al.* (1995) is located near the centre of the chromosome and our marker (BM4440) is near the telomere, it is possible that the potential QTL is closer to the centromere and is too far from our marker to be detected.

In conclusion, we have reported microsatellite markers associated with significant effects for SCS, productive herdlife, milk yield, protein yield, protein percentage, fat yield and fat percentage in the US Holstein population. In addition, we have evidence supporting the location of a QTL on chromosome 21, which affects milk yield, fat yield and protein yield as reported by Ron *et al.* (1994). Our initial findings identifying potential QTL should be confirmed in additional families with other linked markers, using improved statistical methods, and then traced to descendants of these grandsires so that they can be used in marker-assisted selection.

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